Exposure to Grapefruits and Grapefruit Oil Increases Male Mating Success in the Mediterranean Fruit Fly (Diptera: Tephritidae)

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Abstract. Exposure to certain plants or plant compounds may influence the mating success of male fruit flies (Diptera: Tephritidae). Earlier research demonstrated that males of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), exposed to ginger root oil (*Zingiber officiale* Roscoe), bark of the common guava (*Psidium guajava* L.), oranges (*Citrus sinensis* Osbeck) or orange oil obtain significantly more matings than non-exposed (control) males. The purpose of the present study was to determine whether male exposure to another *Citrus* species, the grapefruit *C. paradisi* Macfad., also resulted in increased mating competitiveness of male medflies. Consistent with the data from oranges, males exposed to grapefruits or grapefruit oil had a mating advantage over non-exposed (control) males. In addition, as reported for orange oil, males exposed to grapefruit oil displayed an elevated level of sexual signaling (pheromone-calling), which presumably contributed to their increased mating frequency. The finding that grapefruit, a second *Citrus* species, produced similar effects as oranges suggests that citrus fruits, in general, may enhance the mating performance of male medflies.

Key words: *Ceratitis capitata*, *Citrus paradisi*, mating behavior, insect-plant interaction

Introduction

There is increasing evidence that exposure to certain plants or plant compounds may influence the mating success of male tephritid fruit flies. Studies (Nishida et al. 1988, 1997, Tan and Nishida 1996, Shelly and Dewire 1994, Shelly and Edu 2007) on the oriental fruit fly, *Bactrocera dorsalis* (Hendel), perhaps constitute the most compelling example. Males of this species are highly attracted to methyl eugenol, a compound found in over 30 plant families, which is ingested and subsequently used as a precursor in pheromone synthesis. Males that feed on methyl eugenol or methyl eugenol-bearing plants appear to produce a more attractive pheromone (to females) than methyl eugenol-deprived males and thereby gain a mating advantage.

Exposure to certain plants and plant odors also affects the mating success of males of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). Several studies (e.g., Shelly 2001) have shown that exposure to the aroma of ginger root oil increases mating success of male medflies. Shelly and Villalobos (2004) showed that *C. capitata* males given access to particular sections of branches of the common guava (*Psidium guajava* L.), whose bark contained relatively high amounts of the compound α-copaene, obtained more matings than males denied access to these sites. Papadopoulos et al. (2001) found that male medflies provided access to 'wounded' oranges (with sections of the peel removed, *Citrus sinensis* Osbeck) obtained significantly more matings than males not provided with fruits. These authors also demonstrated that this mating enhancement required direct contact with the fruit: male exposure to wounded oranges covered by a wire screen did not confer a mating advantage. Shelly et al. (2004) confirmed the findings of Papadopoulos et al. (2001) and further showed that exposure of *C. capitata* males to commercially available orange oil

boosted mating success even when direct contact with the oil was prevented. Males exposed to orange oil were observed to signal (i.e., pheromone-call) more actively than non-exposed males, which presumably contributed to their elevated mating success (Papadopoulous et al. 2006).

The purpose of the present study was to determine whether male exposure to another *Citrus* species, the grapefruit *C. paradisi* Macfad., similarly increased the mating competitiveness of male medflies. Trials were conducted that compared the mating success of males exposed to grapefruits or commercially available grapefruit oil to non-exposed (control) males. As shown, treated males had a mating advantage over control males for both types of exposure, and additional work examined whether this result reflected increased pheromone-calling by treated males. Also, previous research (Shelly et al. 2007) involving ginger root oil suggested that increased male mating ability resulted, in part at least, from a more attractive (to females) 'body odor' effected by exposure to the oil's aroma. Accordingly, I used the same assay–involving female approach to freshly killed grapefruit oil-exposed versus non-exposed males–to determine whether the odor of grapefruit oil produced a similar effect.

Materials and Methods

Study insects. Because wild flies were not available in sufficiently large numbers, I used flies from a laboratory colony started with > 300 adults reared from coffee (*Coffea arabica* L.) berries collected near Haleiwa, Oahu. Berries were placed in plastic tubs above a layer of vermiculite, larval development occurred *in situ*, and pupae were sifted from the vermiculite 7–10 d after collection. Emerging adults (and all subsequent generations) were held in screen cages (30 cm cubes) and provided with a sugar-yeast hydrolysate mixture (3:1 v:v), water, and an oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were placed on standard larval diet (Tanaka et al. 1969) in plastic containers over vermiculite for pupation. Flies used in this study were separated by sex within 24 h of eclosion, well before reaching sexual maturity at 5–7 d of age. When tested, flies were 9–10 generations removed from the wild.

Mating trials. Mating trials involving male exposure to grapefruits (Star Ruby variety) were conducted in 2 field cages at the USDA-APHIS facility in Waimanalo, and initial trials involving male exposure to grapefruit oil were conducted in 4 field cages at the USDA-ARS facility, Honolulu. All cages (diameter 3 m, height 2.5 m) were made of nylon-mesh screen and contained 2 artificial trees (*Ficus benjamina* L. type, 2 m tall). Fifty treated males (exposed to grapefruits or grapefruit oil) and 50 control males (non-exposed) were released into each cage at 0900 hrs, and 50 females were released into each cage 15 min later. When tested, males were 7–10 d old, and females were 8–12 d old. For a given test day, treated or control males were chilled and marked with a dot of enamel paint on the thorax (1–2 d before testing). The type of male marked (treated or control) was alternated between successive test days. After release, cages were checked periodically for 3 h, and mating pairs were collected by gently coaxing them into a vial. A total of 12 replicates (cages) involving exposure to grapefruits was conducted over 6 test days, and a total of 16 replicates involving exposure to grapefruit oil was conducted over 4 test days. Mating trials were conducted in March-April, 2009, with air temperature ranging from 23–27°C.

As noted above, treated males were either exposed to grapefruits or grapefruit oil 1 d before testing. For fruit exposure, 4 store-bought grapefruits were rinsed, dried, "wounded" (4–6 small sections [$\approx$ 1 cm^2] of peel were removed, exposing the white inner rind, or albedo), and then placed in screened cages (30 cm cubes), and 60 males were added (along with food and water). These cages were set up at 0830 hrs, and fruits were removed at 1430 hrs. For

exposure to grapefruit oil, 0.5 ml of oil (Citrus & Allied Essences Ltd., Lake Success, NY) was applied to a cotton wick (length 2.5 cm, diameter 1.5 cm) resting in an aluminum foil "boat", which was then placed on the floor of a plastic bucket (volume 5 L) with a screened cover and a sleeved side-opening for moving the flies. Sixty males were then introduced for a 3 h exposure period (0900–1200 hrs). Food and water were placed in the bucket when the oil was removed.

Three additional mating experiments were performed. In the first, treated males were exposed to grapefruit oil as above, except that the oil-laden wick was placed inside a screen mesh container, thus preventing direct contact by the males. Although the observations were not systematic, males exposed to an uncovered wick were rarely seen resting on it. Nonetheless, by preventing contact completely, the role of aroma alone in influencing male mating success could be evaluated more definitively. In another auxiliary experiment, treated males were exposed to grapefruits as above, except that the fruits were placed inside a cotton sock (with the end tied shut), preventing direct contact by males. Note that, because the albedo was left intact, wounding the fruit did not result in any fruit "leakage," consequently the sock covering the fruits was completely dry. In the final experiment, treated males were presented uncovered grapefruits as above but were held 3 d before testing. All auxiliary experiments were conducted at the Waimanalo facility, and a total of 8 replicates was completed over 4 test days for each experiment.

Calling propensity. As described below, exposure to both grapefruits and grapefruit oil increased male mating success. To determine whether this might have reflected increased signaling activity by the treated males, I monitored pheromone-calling of males exposed to grapefruit oil (1 d before testing following the above procedures with an uncovered wick) and non-exposed males in the laboratory. Eight treated or control males (7–10 d old) were placed in glass containers (30 cm cubes with 1 sleeved side for moving flies) situated next to a window at 1000 hrs. Prior to testing, strips of green masking tape were applied to the outer surfaces of the cages to simulate leaves. Then, starting 1 h later, I recorded the number of pheromone-calling and wing-fanning males at 5 min intervals over a 1 h period (n = 13 observations). Four cages–two each with treated and control males, respectively - were observed per day on 8 different days (n = 16 replicates). Cages were placed in the same location every day, and the type of male assigned to a particular cage was alternated between successive test days. Cages were rinsed with water between successive test days. For each cage, I calculated the mean number of males calling per observation and used these values in comparing treated and control males.

Male scent. As noted above, the body scent of male medflies exposed to the aroma of ginger root oil was more attractive to females than that of non-exposed males (Shelly et al. 2007). To determine whether grapefruit oil produced a similar effect, I repeated the protocol used in that earlier study and presented females with freshly killed (by freezing) treated and control males and monitored female presence near them. The use of dead males eliminated the possibility that active displays, behavioral or pheromonal, influenced female response.

On a given test day, 30 females (9–13 d old) were introduced to screen cages (30 cm cubes) at 1000 hrs. Oil-exposed (1 d before testing following the above procedures with an uncovered wick) and non-exposed males (7–12 d old) were then placed in a freezer for 30 min and then held at room temperature for 10 min. Males were then transferred to plastic Petri dishes (5.4 cm diameter) and introduced into the screen cages containing the females. Thus, for all trials, a given cage contained 30 females and 2 Petri dishes (10 cm apart), each holding 10 freshly killed treated or control males.

Starting 1 min after male placement, I recorded the number of females resting on each of the dishes at 1-min intervals over a 30-min period. Because females were not marked,

the number of sightings recorded represented a composite index that encompassed both female arrival and retention on a particular dish. On a given test day, I observed 2 cages simultaneously, which were resting on a table (0.5 m apart) adjacent to a window. The locations (right or left from the observation point) of the 2 treatments within the test cages were alternated between test days to control for a possible position effect. Observations were made on 18 different days for a total of 36 replicates, and the total number of female sightings made per replicate were compared between treated and control males.

Results

Mating trials. In all experiments, males that were exposed to uncovered or covered grapefruits or grapefruit oil and tested 1 d after exposure obtained significantly more matings than non-exposed males (Fig. 1). Results of paired t-tests were: uncovered fruits: $t = 4.7$, $df = 11$, $P < 0.001$; uncovered oil: $t = 4.8$, $df = 15$, $P < 0.001$; covered fruits: $t = 4.9$, $df = 7$, $P = 0.002$; covered oil: $t = 4.0$, $df = 7$, $P = 0.005$). In addition, males tested 3 d after exposure to grapefruits had a mating advantage over control males ($t = 6.5$, $df = 7$, $P < 0.001$, Fig. 1).

Calling propensity. Males exposed to grapefruit oil displayed a significantly higher level of calling than control males. On average, 4.4 ($\pm$ 0.4) treated males were pheromone-calling per observation compared to 3.2 ($\pm$ 0.4) control males ($t = 2.2$, $df = 30$, $P = 0.04$).

Male scent. There was no significant difference in the total number of female sightings made per replicate for treated and control males (14. $\pm$ 1.5 versus 15.7 $\pm$ 1.7 female sightings, respectively, $t = 0.6$, $P = 0.57$, paired t test, $df = 35$).

Discussion

Exposure to grapefruits or grapefruit oil influenced the mating performance of male medflies in a manner similar to that previously described for oranges and orange oil (Papadopoulos et al. 2001, 2006; Shelly et al. 2004). As with *C. sinensis*, exposure to uncovered grapefruits or grapefruit oil increased male mating success. Also, as noted for orange oil (and ginger root oil, Shelly 2001), exposure to covered grapefruit oil increased male mating success. The finding that grapefruits, a second *Citrus* species, produced similar effects as oranges suggests that citrus fruits, in general, may enhance the mating performance of male medflies. Interestingly, *C. capitata* males given access to covered grapefruits still maintained a mating advantage over control males, a finding that contrasts with both oranges and guava bark (Shelly and Villalobos 2004). This represents the first instance where the odor of a natural plant structure (and not a commercially available, botanical oil) alone was found to enhance the mating ability of male medflies.

The enhanced mating competitiveness observed for treated males appears to have resulted, in part at least, from an increased level of sexual signaling. Males exposed to grapefruit oil exhibited a higher level of pheromone-calling than non-exposed males, and this effect, although not tested, presumably characterized male exposure to grapefruits as well. The possibility that exposure to grapefruits or grapefruit oil also resulted in the synthesis of a more attractive pheromonal signal was not addressed, but studies on orange oil (Papadopoulos et al. 2006) and ginger root (Shelly 2001) found no evidence of increased female attraction to the pheromone of treated males over that of control males (independent of any difference in calling level).

Working with ginger root oil, Shelly et al. (2007) showed that *C. capitata* females presented with freshly killed (by freezing) males perched near oil-exposed males more often than control males. Also, females presented with filter paper discs containing hexane extracts (from brief hexane rinses of several males simultaneously) approached extracts

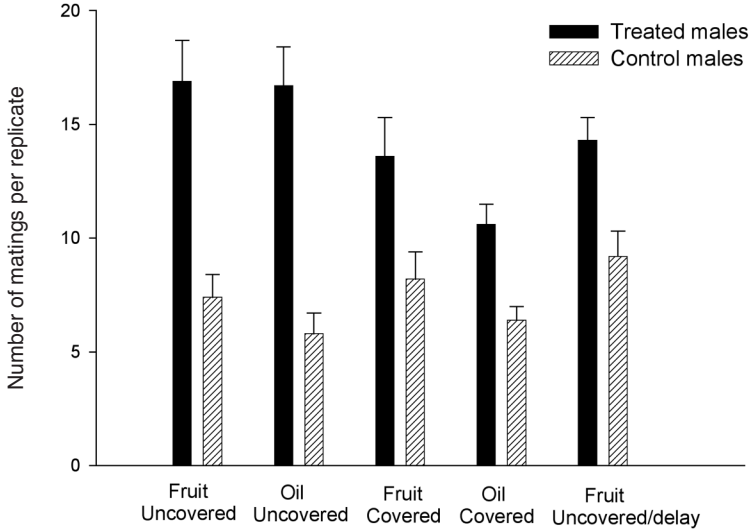

Figure 1. Numbers of matings achieved by treated (fruit- or oil-exposed) and control (non-exposed) males in field-cage trials following exposure to (i) uncovered grapefruits, (ii) uncovered grapefruit oil, (iii) covered grapefruits, or (iv) covered grapefruit oil with trials conducted 1 d after exposure or (v) uncovered grapefruits with trials conducted 3 d after exposure. Bar heights represent means (± SE).

from oil-exposed males more often than extracts from control males. It thus appears that, in addition to increased signaling, males exposed to ginger root oil gained an advantage over non-exposed males via possession of an altered body scent that was more attractive to females. In contrast, despite using the same methods, I found no evidence of such an effect following exposure to grapefruit oil as females were sighted with equal frequency near treated and control males. It thus appears that ginger root oil and grapefruit oil have differing effects on the exoskeleton (and body scent) of male medflies and that exposure to grapefruit oil (and possibly other oils) may boost male mating success independent of any effect on male body odor.

The identity of the plant compound(s) responsible for the increased mating ability of male medflies is not known with certainty. Shelly (2001) showed that the hydrocarbon sesquiterpene α-copaene alone enhances the mating success of *C. capitata* males, and oranges (Teranishi et al. 1987) and orange oil (Shelly et al. 2004), grapefruits (Dou 2003) and grapefruit oil (S. Young, personal communication), ginger root oil (Takeoka et al. 1990), and guava bark (Shelly and Villalobos 2004) all contain this compound. However, whether other compounds act synergistically with α-copaene remains unknown.

Based on the results from orange oil (Shelly et al. 2004) and ginger root oil (Shelly and McInnis 2001), it appears likely that grapefruit oil would also increase the mating success of mass-reared, sterile male medflies. Several programs utilizing the Sterile Insect Technique (SIT) against the medfly (e.g., Los Alamitos, USA; Retalhuleu, Guatemala) have incorporated pre-release exposure of sterile males to ginger root oil as part of their standard operating procedure. If grapefruit oil were found to be effective on a large scale, substituting it for ginger root oil would reduce costs. At present, ginger root oil costs $79/kg compared to only $30/kg for grapefruit oil (L. Milack, personal communication). While the savings would be

small relative to the overall expense associated with an SIT program (e.g., the Los Alamitos program uses only 1 kg of ginger root oil per month), a shift to grapefruit oil may nonetheless deserve consideration in the face of increasingly restrictive operating budgets.

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Literature Cited

Dou, H. 2003. Volatile differences of pitted and non-pitted 'Fallglo' tangerine and whie 'Marsh' grapefruit. Hortscience 38: 1408–1409.

Nishida, R., K.H. Tan, M. Serit, N.L. Lajis, A.M. Sukari, S. Takahashi, and **H. Fukami.** 1988. Accumulation of phenylpropanoids in the rectal glands of males of the Oriental fruit fly, *Dacus dorsalis*. Experientia 44: 534–536.

Nishida, R., T.E. Shelly, and **K.Y. Kaneshiro.** 1997. Acquisition of female-attracting fragrance from a Hawaiian lei flower, *Fagraea berteriana*, by males of the Oriental fruit fly. J. Chem. Ecol. 23: 2275–2285.

Papadopoulos, N.T., B.I. Katsoyannos, N.A. Kouloussis, and **J. Hendrichs.** 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata*. Entomol. Exp. Appl. 99: 253–261.

Papadopoulos, N. T., T.E. Shelly, N. Niyazi, and E. Jang. 2006. Olfactory and behavioral mechanisms underlying enhanced mating competitiveness following exposure to ginger root oil and orange oil in males of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). J. Insect Behav. 19: 403–418.

Shelly, T.E. 2001. Exposure to α-copaene and α-copaene-containing oils enhances mating success of male Mediterranean fruit flies. Ann. Entomol. Soc. Am. 94: 497–502.

Shelly, T.E. and **A.M. Dewire.** 1994. Chemically mediated mating success in male oriental fruit flies, *Bactrocera dorsalis* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 87: 375–382.

Shelly, T.E. and D.O. McInnis. 2001. Exposure to ginger root oil enhances mating success of irradiated, mass-reared males of Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 94: 1413–1418.

Shelly, T.E. and **E.M. Villalobos.** 2004. Host plant influence on the mating success of male Mediterranean fruit flies: variable effects within and between individual plants. Anim. Behav. 68: 417–426.

Shelly, T. E. and **J. Edu.** 2007. Exposure to the ripe fruit of tropical almond enhances the mating success of male *Bactrocera dorsalis* (Diptera: Tephritidae). Fla. Entomol. 90: 757–758.

Shelly, T.E., C. Dang, and **S. Kennelly.** 2004. Exposure to orange (*Citrus sinensis* L.) trees, fruit, and oil enhances mating success of male Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]). J. Insect behave. 17: 303–315.

Shelly, T.E., J. Edu, E. Pahio, and **J. Nishimoto.** 2007. Scented males and choosy females: does male odor influence female mate choice in the Mediterranean fruit fly? J. Chem. Ecol. 33: 2308–2324.

Takeoka, G., R.A. Flath, T.R. Mon, R.G. Buttery, R. Teranishi, M. Guntert, R. Lautamo, and **J. Szejtli.** 1990. Further applications of permethylated β-cyclodextrin capillary gas chromatographic columns. J. High Resol. Chromatogr. 13: 202–206.

Tan, K.H. and **R. Nishida.** 1996. Sex pheromone and mating competition after methyl eugenol consumption in the *Bactrocera dorsalis* complex, pp. 147–153. *In* B. A. McPheron and G. J. Steck (eds.), Fruit fly pests: a world assessment of their biology and management. St. Lucie Press, Delray Beach, FL.

Tanaka, N., L.F. Steiner, K. Ohinata, and **R. Okamoto.** 1969. Low-cost larval rearing medium for mass production of oriental and Mediterranean fruit flies. J. Econ. Entomol. 62: 967–968.

Teranishi, R., R.G. Buttery, K.E. Matsumoto, D.J. Stern, R.T. Cunningham, and **S. Gothilf.** 1987. Recent developments in the chemical attractants for tephritid fruit flies. ACS Symp. Ser. 330: 431–438.